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The variability in activity of the universally expressed human cytomegalovirus immediate early gene 1 enhancer/promoter in transgenic mice

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ABSTRACT

Transcriptional control regions which direct transgene expression to all tissues in transgenic animals can be useful tools for gain-of-function experiments in transgenic animals. A candidate for this purpose is the regulatory region of the human cytomegalovirus immediate early 1 gene (HCMVIE1) which is highly expressed in many lines of tissue culture cells. Here we analyzed the activity of the HCMVIE1 enhancer/promoter using a sensitive reporter gene, the bacterial chloramphenicol acetyltransferase (CAT) gene. Three lines of transgenic mice with an intact transgene were established. All 3 lines expressed the transgene in all 28 tissues analyzed; however, levels of expression between the three lines varied up to 100,000 fold. In addition, expression levels in the high expressing line varied over a 10,000 fold continuum, while expression levels between tissues was almost uniform in the lowest expressing line. The transgene was well expressed in the high expressing line; CAT activity in the highest expressing tissues was equivalent to levels previously reported for tissue specific CAT transgenes active only in a limited number of tissues. These data support the utilization of the HCMVIE1 enhancer/promoter as a means of expressing a transgene in all tissues, but indicate that lines with substantially different overall levels of expression may be generated, and that markedly different levels of tissue specific expression may be found when the overall level of transgene expression is high.

INTRODUCTION

The enhancer/promoter of the HCMVIE1 gene demonstrates high transcriptional activity in virtually every tissue culture cell (1, 7, 19, 20). This high level of expression in a broad range of tissue culture cells is due to the interaction of unique and repeated sequence motifs in the enhancer with transcription factors from the NF-κB, NF1, SP1, CREB and MDBP families (2, 6, 8, 9, 10, 26, 27). Because of these properties, the HCMVIE1 promoter/enhancer frequently has been used to transiently express genes in different cell types (18). It has been employed less often in stable expression studies in cell lines (10) or transgenic animals (3, 15, 17, 25).

Transgenic mice can be used for dominant gain-of-function experiments in which the effects of specific gene products are examined in tissues in which they are not normally found (22), or when their expression is deregulated (11). Because there are few characterized promoters which are active in a broad range of tissues in transgenic animals we analyzed the ability of the HCMVIE1 promoter/enhancer to express a transgene in the differentiated tissues of transgenic mice.

MATERIAL AND METHODS

Recombinant DNA

The hybrid gene injected into oocytes contained HCMVIE1 sequences between −524 and +55, the bacterial chloramphenicol acetyltransferase (CAT) gene and the SV40 early polyadenylation site contained in an SV40 fragment which includes the small t intron (Figure 1). This hybrid gene was functional and highly expressed in different cell lines (19, 20) and *in vitro* (7).

Transgenic mice

Zygotes from FVB/N inbred genetic background were microinjected according to standard procedures with a 2.3 kb *Hin*dIII-*Kpn*I fragment (3 μg/ml in 10 mM Tris-HCl at pH 7.5, 0.1 mM EDTA) containing the hybrid gene. The prokaryotic cloning vector sequences were separated from the microinjected fragment by agarose gel electrophoresis followed by purification of the DNA from the gel matrix using the glass-powder procedure.

Analysis of chloramphenicol acetyltransferase activity

Tissues were harvested from six heterozygotic and two homozygotic F3 transgenic mice from line 283, from one heterozygotic F3 transgenic mouse from line 200, and from two heterozygotic F3 transgenic mice from line 266. The same section and amount of tissue from each organ was processed from each animal. Protein extracts were made from the tissues indicated in Figure 2 and CAT assays performed as previously described

(14). CAT activity was consistently measured within the linear range by varying the amount of protein extract used between 5 μg and 100 μg and varying the incubation times between 15 minutes and 6 hours at 37°C. CAT activity was quantitated by either scintillation counting or radioanalytic imaging (AMBIS) of the unacetylated and acetylated forms of chloramphenicol and% acetylation was calculated.

RESULTS AND DISCUSSION

Production of transgenic mice

The hybrid gene was injected into 970 zygotes, and from the resulting 150 liveborn pups we were able to establish only 3 lines with an intact transgene. Southern blot analysis revealed that each of these lines contained less than 10 copies of the intact HCMVIE1 transgene (data not shown). Nine lines of transgenic mice with completely rearranged transgenes were identified but not analyzed further. The low efficiency of obtaining transgenic mice with this construct is in contrast to our experience with many other gene constructs. Since a similar observation has been made with an HCMVIE1 transgene containing the *lacZ* reporter gene (3), this suggests that the HCMVIE1 regulatory region may be subject to rearrangement or deletion or may even be lethal when integrated at many sites in the host genome.

Variability of CAT expression between different lines

All three lines of transgenic mice expressed the transgene in all tissues analyzed (Figure 2). Two significant observations can be made from comparing the levels of expression in the different tissues from each line. First, the overall level of expression was markedly different in each line. The highest expressing tissues in the high expressing line 283 expressed CAT an average of 100-fold higher than line 200 and at levels up to 100,000-fold higher than line 266. Second, expression levels from the transgene in different tissues were almost uniform in the low expressing line 266 but varied over a 10,000 fold range in the high expressing line 283. These differences between lines most likely reflect effects of the site of integration on expression. As the HCMVIE1-*lacZ* transgene demonstrated the same two phenomena, markedly different expression levels between lines and a large range of expression levels in specific tissues only

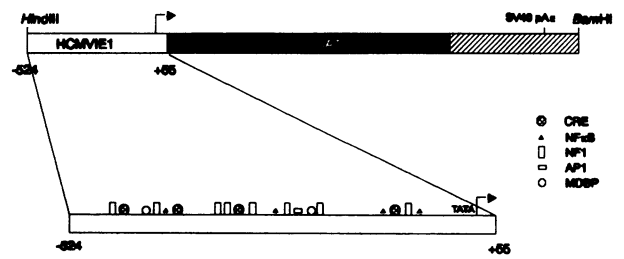

Figure 1. Structure of the HCMVIE1 enhancer/promoter transgene. The numbers indicate nucleotide positions. Binding sites for known transcription factors are shown. The position of the TATA box is marked and the arrow points to the transcriptional start site. The transgene contains the SV40 early polyadenylation site which lies within an SV40 fragment which includes the small t intron. Abbreviations used are: HCMVIE1: human cytomegalovirus immediate early gene 1; CAT: chloramphenicol acetyltransferase; SV40 pA_E: SV40 virus early polyadenylation site; CRE: cyclic AMP response element; NFκB: NF-κB binding site; NF1: nuclear factor 1 binding site; AP1: AP1 binding site; MDBP: methylation dependent binding protein binding site.

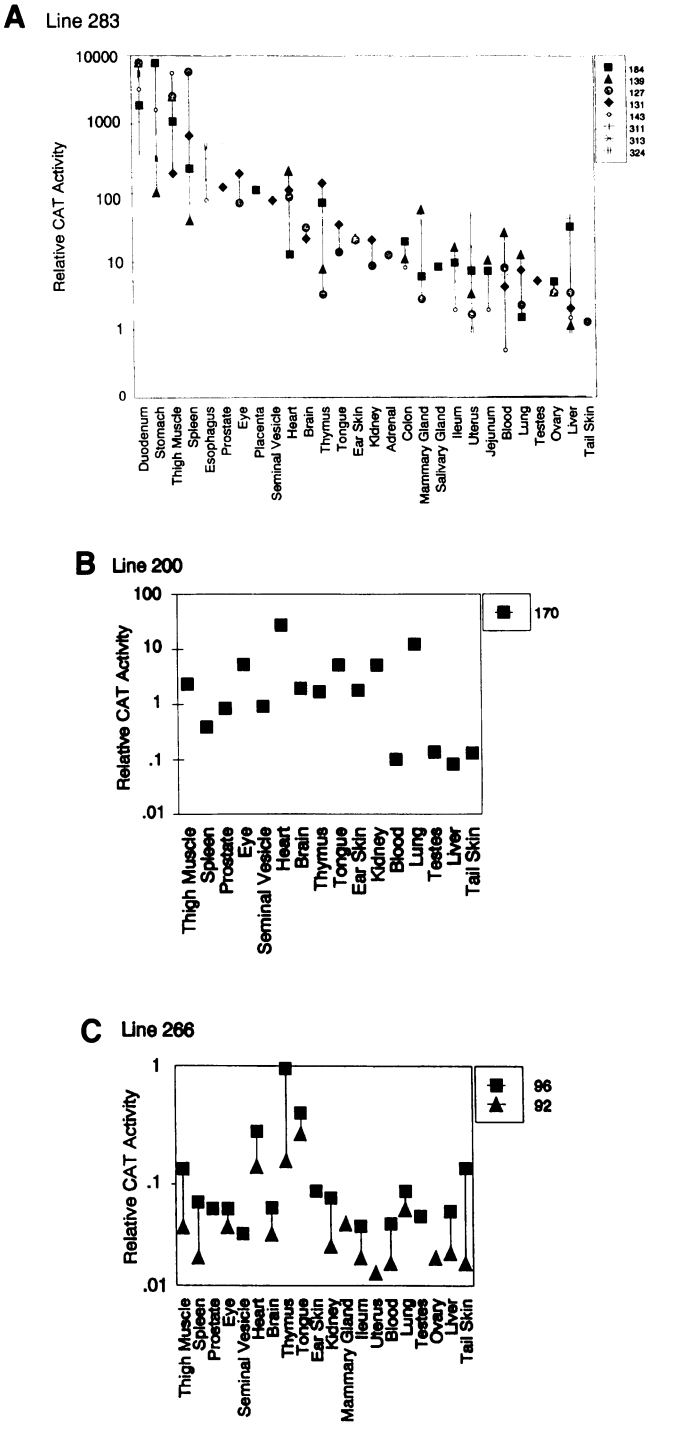

Figure 2. CAT activity in the tissues of transgenic mice from lines 283 (A), 200 (B) and 266 (C). The symbol corresponding to the identification number of each mouse is listed at the upper right of each graph. The exact number of each tissue type analyzed is indicated by the symbols. All data is presented on a log scale. The relative CAT activities directly reflect the amount of enzymatic activity which would be present in an extract containing 100 μg cytoplasmic protein incubated for 1 hour at 37°C. For example, 1 corresponds to 1% acetylation and 10 corresponds to 10% acetylation measured under the conditions described above. The CAT activities in the highly expressing tissues from line 283 were extrapolated from direct measurements of% acetylation between 5 and 25 μg cytoplasmic protein incubated for 15 to 60 min. at 37°C to keep the measurement of CAT activity within the linear range. The sex (M or F), age (in months) and whether the individual mice were heterozygous (HE) or homozygous (HO) is as follows: Line 283: 184: F,2m,HE; 139: F,10m,HE; 127: F,7m,HE; 131: M,6m,HE; 143: M,10m,HE; 311: F,4m,HO; 313: F,4m,HO; 324: F,2m,HE. Line 200: 170: M,6m,HE. Line 266: 96: M,6m,HE; 92: F,6m,HE.

in a high expressing line (3), this pattern of expression may be seen with other transgenes containing this enhancer/promoter.

The observation that the chromatin domain surrounding the HCMVIE1 transgene exerts a dominant effect on the transgene's level of activation is consistent with results from virtually every transgenic experiment. However, the fact that only the highest expressing line demonstrates great variation in expression levels between different tissues suggests that the interaction of transcription factors with chromatin in this integration site was fundamentally different than their behavior in the other two integration sites. In addition, while in other transgenic experiments it is not uncommon to identify lines of transgenic animals with an intact transgene but no detectable expression (22), in this case, all lines expressed the transgene. One can speculate that there is a relative hierarchy in the interaction of transcription factors and/or transcription complexes with chromatin. Some may require a permissive chromatin domain to function fully but others can stimulate transcription even in more constrictive domains. In addition, because the lower expressing lines were also associated with less tissue to tissue variability, these observations suggest that the tissue specific potential of an enhancer/promoter may only be revealed when it is integrated into permissive chromatin.

The fact that all three lines of transgenic mice expressed the transgene in this study while in a previous report only 2/5 lines expressed the transgene (25) may be due to the assay system employed. The levels of expression that we detected with the CAT assay may have been below the level of detection for an RNase protection assay.

Finally, the lack of methylation sensitive restriction enzyme sites in this enhancer/promoter construct make it difficult to assess what role, if any, methylation plays in this integration site dependent expression pattern.

Tissue specific activity of the HCMVIE1 enhancer/promoter

Comparison of our data with both previously published (25) and unpublished (3) data revealed that all three different types of HCMVIE1 transgenes were highly expressed in the heart, stomach and spleen. Since the levels of CAT activity in line 283 were equivalent to those reported with tissue specific enhancer/promoters linked to the CAT gene (15, 16, 21, 23, 24), this suggests that an HCMVIE1 construct may be useful for experiments in which high levels of expression are desired in these tissues.

The high expression in thigh muscle measured in both line 283 presented here and in the *lac*Z transgene (3) contrasts with the lack of detectable expression in a previous report (25). This is important to comment upon as the high expression found in a HCMVIE1 transgene which included intron A of the HCMVIE1 gene was attributed to a possible muscle specific enhancer located in that intron (17). Because neither our construct or that with the *lac*Z transgene contained this intron, it is clear that the reported putative muscle specific enhancer sequence is not necessary for high expression in skeletal muscle.

One other conclusion that can be made from comparing the expression levels in various tissues from the different lines reported here and those from different transgenes (3, 25) is that expression from the HCMVIE1 enhancer/promoter in the liver was always low. As many of the transcription factors known to stimulate the HCMVIE1 enhancer, such as NF-1 and NF-κB have been isolated from the liver, this suggests that the integrated HCMVIE1 enhancer/promoter is repressed or cannot be accessed by these transcription factors in differentiated liver. Modification of these transcription factors in liver tissue cannot be excluded. In contrast, the murine CMVIE1 enhancer/promoter which contains many of the same cis-acting elements was active in transiently transfected hepatic tissue culture cells (5). The low expression in mouse liver is not an artifact of the CAT assay as no CAT mRNA could be detected on Northern blot analyses (data not shown) and CAT activity was readily measurable in the liver of transgenic mice expressing a mutant polyoma promoter CAT construct (13).

Variability of expression within line 283

The variability of expression between individual mice was examined using line 283 because the differences in tissue specific activity were greatest in this line (Figure 2A). Two important features emerged from these comparisons. First, there was variation in expression levels in specific tissues between individual animals, but there is a general trend from high to low expressing tissues. No obvious correlations with sex, age, maternal or paternal inheritance or heterozygosity or homozygosity can be found to explain the animal to animal variation (See legend to Figure 2A). As the same experimental procedures were followed throughout, the data reflects the degree of animal to animal variation in expression as measured by a CAT assay.

In conclusion, the HCMVIE1 enhancer/promoter appears to be most useful for experiments in which transgene expression in all tissues is desired and when some variation in expression levels from tissue to tissue is acceptable. This might be true when the dose effect of a transgene as well as its effect in different tissues needs to be determined. The data suggests that this enhancer/promoter could be useful to express genes at high levels in heart, stomach, spleen and thigh muscle. Additional experiments using different reporter genes will further define the tissue specific activity of this promoter. Finally, these experiments illustrate the important provision that the function of transcription factors or complexes on a promoter can vary greatly from one chromatin domain to another.

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